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Phenotypic and genotypic traits of vancomycin resistant enterococci from healthy food producing animals

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Contents

Phenotypic and genotypic traits of vancomycin resistant enterococci from healthy food producing animals

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Abstract

Background: Food producing animals may be a reservoir of vancomycin resistant enterococci (VRE) affecting veterinary and human medicine, either by horizontal transfer of resistance genes, or by clonal dissemination of strains. The aims of this study were to estimate the current occurrence of VRE among healthy cattle, pigs, poultry, and sheep in Switzerland, and to characterise phenotypic and genotypic traits of the isolates.
Methods: Caecal content from slaughtered cattle, pigs and sheep, and pooled faecal matter from poultry flocks at slaughter were cultured for VRE. Species identification and vancomycin genotyping were performed for all isolates. Antimicrobial resistance profiling, conjugation experiments and whole genome sequencing were carried out on a subset of the isolates.
Results: VRE was isolated from 2% of bovine and 3% of porcine samples, and from 16% of the poultry flock samples. All isolates harboured the *vanA* gene. Twenty-three isolates (one *Enterococcus* (*E.) faecalis* from cattle, six *E. faecium* from pigs, six *E. faecium* and 10 *E. durans* from poultry were further analysed. All porcine vancomycin resistant *E. faecium* (VRE*fm*) isolates belonged to sequence type (ST)133, the majority thereof were resistant to penicillin (PEN) and tetracycline (TE). VRE*fm* from poultry belonged to ST310, ST157 and ST13, and the majority was resistant to erythromycin (ERY). Most of the *E. durans* was resistant to ERY/TE. Conjugal transfer of *vanA* to human *E. faecalis* strain JH2-2 was observed for porcine isolates only. Three different types of Tn*1546*-like elements carrying the *vanA* operon were identified among the isolates. Type I corresponding to the prototype Tn*1546*-like element (identical to GenBank M97297) was detected in four *E. durans* strains from poultry and one vancomycin resistant *E. faecalis* (VRE*fc*) from cattle. Type II carried the G8234T nucleotide point mutation in *vanX* and was the most prevalent structure (identified in 14 strains). Type III contained a reverse insertion (IS) element IS1*252* upstream of *vanR*, and was observed exclusively in *E. durans*.
Conclusions: Among food producing animals we detected VRE belonging to STs, and harbouring Tn*1546*-like variants that have been found previously in food producing animals, in healthy and diseased humans, but not associated with nosocomial outbreaks.

Keywords

Enterococcus faecium, *E. faecalis*, *E. durans*, *vanA*, whole genome sequencing, *Tn1546*, cattle, pigs, poultry

Zusammenfassung

Ziel dieser Studie war es, das Vorkommen von Vancomycin-resistenten Enterokokken (VRE) in Nutztieren in der Schweiz zu eruieren, sowie die phäno- und genotypischen Eigenschaften der Isolate zu charakterisieren. Hierfür wurden Proben aus dem Zäkum geschlachteter Rinder, Schweine, Schafe und Mastgeflügelherden entommen und auf das Vorkommen von VRE hin untersucht. Bei allen nachgewiesenen VRE-Isolaten wurden eine Speziesidentifikation und Vancomycin-Genotypisierung durchgeführt. Von einem Teil der Isolate wurden Resistenzprofile erstellt und Konjugationsexperimente sowie eine Gesamtgenomsequenzierung durchgeführt. VRE wurden aus 2% der bovinen und 3% der porcinen Proben isoliert, sowie aus 16% der Geflügelherden. Alle VRE-Isolate waren Träger des *vanA* Gens. 23 Isolate wurden weiter untersucht. Alle porcinen Vancomycin-resistenten *E. faecium* (VRE*fm*) Isolate gehörten zum Sequenztyp (ST)133, davon war der grösste Teil resistent gegenüber Penicillin (PEN) und Tetracyclin (TE). VRE*fm* von Geflügelherden gehörten zu ST310, ST157 und ST13, diese waren grösstenteils resistent gegenüber Erythromycin (ERY). Die meisten *E. durans* waren resistent gegenüber ERY/TE. Nur in den porcinen Isolaten konnte ein Gentransfer via Konjugation von *vanA* auf den humanen *E. faecalis*- Stamm JH2-2 beobachtet werden. Unter den Isolaten wurden drei verschiedene Typen der Tn*1546*-like Transposons als Träger des *vanA* Operons identifiziert.

Schlüsselwörter: *Enterococcus faecium*, *E. faecalis*, *E. durans*, *vanA*, Gesamtgenomsequenzierung, *Tn1546*, Rind, Schwein, Geflügel

Introduction

Antimicrobial resistance has now become a permanent aspect of human medicine with vancomycin resistant enterococci (VRE) gaining importance as nosocomial pathogens worldwide [1]. The World Health Organization (WHO) ranks vancomycin resistant *E. faecium* (VRE*fm*) as a pathogen of high priority in its global list of important antibiotic-resistant bacteria [2]. For European countries, population-weighted mean percentage of resistance to vancomycin in invasive VRE*fm* increased from 10.5% in 2015 to 17.3% in 2018 [3]. By contrast, in *E. faecalis*, vancomycin resistance remains infrequent in Europe [3].

Nosocomial VRE*fm* may arise through independent events of introduction and subsequent dissemination within hospitals, but are also thought to generate within patients under antimicrobial therapy, most probably by the acquisition of resistance genes by means of horizontal gene transfer (HGT) [4, 5, 6, 7]. One of the most important genetic determinants of vancomycin resistance is represented by the *vanA* gene cluster, which is organised as an operon consisting of seven open reading frames, and is typically associated with transposons such as Tn*1546* [8, 9]. Tn*1546*-type transposons play a key role in the acquisition and dissemination of vancomycin resistance among enterococci [9, 10]. Tn*1546* transposons vary structurally, on account of point mutations, deletions or the presence of insertion sequence (IS) elements [11, 12]. These variations provide potential markers to type and trace the spread of *vanA* genes among enterococci isolated from different sources [13, 14, 15].

Most human clinical VRE*fm* strains belong to the *E. faecium* lineage designated Clade A1 [16, 17]. This clade contains the vast majority of strains isolated from clinical settings, including isolates belonging to clonal complex (CC)17 [17, 18], and to the recently emerged sequence types (STs)203 and ST796 [4, 19, 20, 21]. Clade A2 contains strains that are predominantly associated with sporadic human infections and with livestock [18].

The proliferation of VRE in livestock in Europe is attributed to the past use of avoparcin, which was introduced in 1975 as a growth promoter, but which confers cross-resistance to vancomycin [22]. The EU ban on antimicrobial growth promoters enforced in 2006 (EC no. 1831/2003) lead to a decline of the prevalence of VRE among farm animals [22]. Nevertheless, VRE continues to be readily detected in samples from livestock when using selective media, and its persistence is suggested to be maintained by co-selection , i.e., the use of macrolides, tetracycline, or copper, or by the presence of plasmid addiction systems [22]. Accordingly, food producing animals may be considered a reservoir of VRE that affects veterinary and human medicine, either by horizontal transfer of vancomycin resistance genes between animal and human adapted enterococci, or by clonal dissemination of resistant strains [22].

This study aimed to assess the prevalence of VRE among healthy cattle, pigs, poultry, and sheep at slaughter and to characterise and compare phenotypic and genotypic traits of the isolates from these different sources.

Material and Methods

Sampling and bacterial isolation

For slaughter cattle (n=362), pigs (n=350), and sheep (n=218), swab samples were aseptically collected on 14 different days from caecal contents by cutting through the caecal wall with sterile scissors. Each caecum was swabbed once, avoiding an overload of fecal matter. Swabs were placed in sterile stomacher bags and transported to the laboratory. Animal and herd identification were collected along with each sample.

For poultry samples, fecal matter was collected on 9 different days at the entry of a poultry slaughterhouse from the crates of 102 poultry flocks (approximately 6,000 chicken per flock).

Pooled samples were placed in sterile bags and flock identification was noted for each sample.

In the case of caecum samples, the excess lengths of the swabs were broken off, and 20ml brain heart infusion (BHI) broth with 6.5% NaCl (Oxoid, Pratteln, Switzerland) was added to each bag. For poultry samples, broth was added directly to each bag. Samples were homogenised for 30-60' using a stomacher and incubated for 18-24h at 37°C. From the pre-enrichment broth, one loopful was streaked onto VRE select agar (BioRad, Cressier, Switzerland) and incubated for 48h at 37°C.

From samples with presumptive enterococci positive colonies, colonies of different colony morphology were selected and purified on sheep blood agar (BioRad). In case of unclear identity, isolates were tested for catalase activity and catalase negative isolates were further analysed.

Species identification was performed by matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF-MS, Bruker Daltonics, Billerica, MA, USA) using Compass FlexControl version 3.4 software with the Compass database version 4.1.80.

All isolates were stored in 20% glycerol at -20°C for further analysis.

Multiplex PCR detection of *vanA*, *vanB*, *vanC-1*, and *vanC-2/3* genes

Bacterial DNA was prepared by a standard lysis and boiling method [23]. Multiplex PCR targeting *vanA*, *vanB*, *vanC-1*, and *vanC-2/3* genes was carried out on all isolates using custom synthesised primers (Microsynth, Balgach, Switzerland) and conditions published previously [24]. Amplicons were visualised under ultraviolet light after electrophoresis in 1% agarose gel stained with ethidium bromide. *E. faecalis* ATCC51299 (*vanB*) and *E. casseliflavus* (*vanC*) [25] were used as positive controls.

Antimicrobial susceptibility testing

Determination of the minimal inhibitory concentrations (MICs) of vancomycin was performed using a gradient strip (Etest, Biomérieux, Geneva, Switzerland) according to the manufacturer's instructions. Antimicrobial susceptibility profiling was performed using VITEK 2 Compact system with AST-GP69 cards (BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The MIC values were interpreted according to the CLSI susceptibility breakpoints, where available [26]. Antimicrobials with existing CLSI breakpoints included ampicillin (AMP), amoxicillin/clavulanate (AMC), erythromycin (ERY), penicillin G, (PEN) and tetracycline (TE).

Conjugation experiments

Transfer experiments were performed using a modified solid mating protocol and *E. faecalis* JH2-2 (rifampicin resistant, vancomycin susceptible) as a recipient [27]. In brief, 40µl volumes of overnight cultures of donor and recipient cells grown in BHI broth were mixed, concentrated by centrifugation, and resuspended in 20µl of BHI broth. The mixture was dispensed onto BHI agar plates and incubated at 37°C for 18-20 hours. One loopful of cells were collected in 400 µl BHI broth. Serial dilutions were plated on BHI supplemented with 6 mg/L vancomycin and 50 mg/L rifampicin. The resulting transconjugants were purified and subjected to identification by MALDI-TOF-MS.

Conjugation frequency was expressed as number of transconjugants per recipient cell.

Transconjugants were tested by singleplex PCR to confirm the presence of *vanA* using primers and conditions described previously [24].

Whole genome sequencing

Bacterial cultures were grown over night in 5 ml BHI with 6.5% NaCl. Chromosomal DNA was isolated from 1 mL overnight cultures using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Sequencing was done on an Illumina MiniSeq (Illumina, San Diego, CA, USA) and reads were assembled using Spades 3.12.0 [28] and Shovill [29], as described previously [30]. Species were identified using SpeciesFinder2.0 [31] available at https://cge.cbs.dtu.dk/services/SpeciesFinder/.

Draft genomes were annotated using Prokkka using standard settings [32]. Resistance genes were identified using the resistance gene identifier RGI from the comprehensive antibiotic resistance database [33, 34]. Searches were performed with standard setting and performed locally against the CARD database downloaded in September 2019.

Variations of the Tn*1546* element were identified based on what is considered the Tn*1546* prototype (GenBank M97297) [15, 35]. Tn*1546* sequences were compared by using average nucleotide identity (ANI). The ANI was calculated according to Richter et al [36] using the python script PyANI.py [37].

An ANI-base tree was constructed using an in-house R script. Shortly, the Euclidian distance in the relative-identity matrix produced by PyANI.py. was calculated using the function “dist” from the package cluster [38] und subsequently clustered using the function “hclust”.

Core-genome multilocus sequence typing (cgMLST) was performed in the software package SeqSphere 4.1.9 (Ridom, Münster, Germany), and the *Enterococcus faecium* MLST database (https://pubmlst.org/efaecium/) and the *Enterococcus faecalis* MLST database (https://pubmlst.org/efaecalis/), respectively, to determine sequence types (STs).

Alignments of the *vanA*-operon were visualised using CLC Main Workbench 8.1.3.

Results

Prevalence of VRE among healthy food producing animals

Overall, VRE were isolated from six (2%) of the cattle samples, 12 (3%) of the swine samples, and 16 (16%) of the poultry flocks. No VRE were isolated from sheep samples, and the six cattle samples were restricted to one herd. A total of 34 *vanA* positive enterococci isolates were identified. No *vanB*, *vanC-1*, or *vanC-2/3* genes were detected.

A subset of 23 strains were selected for further analysis. To avoid sample clustering, isolates from different cattle and pig herds were taken. Strains included *E. faecalis* isolated from cattle (n=1), *E. faecium* isolated from pigs (n=6), *E. faecium* (n=6) and *E. durans* (n=10) isolated from poultry. Taken together, 12 *E. faecalis*, 10 *E. durans*, and one *E. faecalis* were analysed in this study.

AST and detection of resistance genes

All isolates exhibited MICs of vancomycin of > 32 µg/ml (Table 1). Susceptibility profiling for additional antimicrobials with existing CLSI breakpoints showed that among the *E. faecium* isolated from pigs, the resistance phenotype PEN/TE represented the most common pattern (83%), followed by ERY/PEN/TE (17%).

Among the *E. faecium* from poultry, ERY (67%) predominated over PEN (17%), and one isolate remained susceptible. For *E. durans* isolates, the resistance pattern ERY/TE was observed in 70%, while TE was found in 30% of the isolates, respectively.

The *E. faecalis* isolated from cattle did not exhibit an additional resistance phenotype.

Other antimicrobials included in the Vitek 2 panel to which enterococci are intrinsically resistant, or for which no breakpoints are available had MIC values that are listed in Additional file 1.

Regarding the resistance genotype, aminoglycoside resistance genes were detected among 22 (95.7%) of the isolates, corresponding to all the *E. faecium* and *E. durans* strains and excluding the *E. faecalis* isolate. Tetracycline resistance genes were observed uniformly among *E. faecium* from pigs, in eight (80%) of the *E. durans,* but were absent in *E. faecium* for poultry and in the *E. faecalis* isolate (Table 1). Erythromycin genes were detected in eight (80%) of the *E. durans* strains. Notably, the lincosamide, streptogramin A and pleuromutilin (LS_AP) resistance gene *eat(A)$_v$* was identified uniformly in *E. faecium* from pigs and from poultry (Table 1).

Genes potentially conferring metal-resistance including cadmium resistance genes *cad(A)* and *cad(C)*, the copper resistance gene *cop(Z)*, the metal transport repressor gene *czr(A)*, mercury resistance genes *mer(A)* and *mer(R)*, and the putative zinc transporter gene *zos(A)*, were detected exclusively in isolates from pigs.

Mating Experiments

Sixteen strains, including one *E. faecalis* from cattle, six *E. faecium* from pigs, six *E. faecium* from poultry, and three *E. durans* from poultry were selected as donors for conjugative transfer to the recipient *E. faecalis* JH2-2. Transconjugants were obtained from five donors, all of which were *E. faecium* isolates from pigs. Vancomycin resistance was transferred with frequencies of 1.7×10^{-7} (donor Sw245), 4.3×10^{-7} (donor Sw253), 5×10^{-6} (donor Sw290), 2×10^{-6} (Sw292), and 1.5×10^{-5} (Sw342), per recipient, respectively.

Characterization of the Tn*1546* structures

Analysis of the Tn*1546* structures distinguished three different Tn*1546*-like types I-III, respectively (Table 1). A cluster dendrogram showing the Tn*1546* types of all the isolates analyzed in this study and the prototype Tn*1546* (M97297) is presented in Figure 1.

The structure of the *van* operon in type I was identical to the prototype described previously (GenBank M97297), and included four *E. durans* isolates from poultry and the *E. faecalis* isolate from cattle (Table 1).

Type II Tn*1546*-like elements carried a G to T point mutation in *vanX* at position 8234 of the *van* operon and were identified in the six *E. faecium* from poultry, in the six *E. faecium* from pigs, and in two *E. durans* isolates from poultry (Table 1 and Figure 1). The *Tn1546*-like type II elements identified in *E. faecium* from poultry contained an *aadK* gene downstream of *vanZ*, whereas those found in pigs carried *mer(A)* and those detected in *E. durans* contained a topoisomerase gene. Examples of type II Tn*1546*-like elements are shown in Figure 2.

Finally, type III Tn*1546* was identical to the type II structure, but disrupted by IS*1252* in the *orf*2-*van*R intergenic region. Type III elements were detected in four *E. durans* isolates from poultry and carried a topoisomerase gene located downstream of *vanZ* (Figure 2).

Multilocus sequence typing

MLST analysis of the 12 VRE*fm* revealed the occurrence of four different STs. The most frequent ST was ST133, which was found in the six isolates from pigs. ST310 was identified in three, ST157 in two, and ST13 in one isolate from poultry, respectively (Table 1).

MLST further assigned the *E. faecalis* from cattle to ST29 (Table 1).

Accession numbers

The whole genome shotgun sequences have been deposited at GenBank numbers VYUB00000000 to VYUX00000000.

Discussion

The use of antimicrobials as growth promoters was banned by law in Switzerland in 1999 [39]. During the decades that followed, VRE was detected at very low levels in the context of resistance monitoring of livestock [40]. However, between 2013 and 2016, one *E. faecalis* isolate from cattle, one *E. faecalis* from broilers, and two *E. faecium* isolates from fattening pigs were resistant to vancomycin, indicating that VRE are present in food animals in Switzerland [40, 41].

The present study demonstrates the occurrence of *vanA*-type *E. faecalis*, *E. faecium* and *E. durans* among Swiss cattle, pigs, and poultry flocks 20 years after the ban on avoparcin use. The persistence of VRE in the absence of an obvious selective pressure has been observed previously and is thought to be a consequence of co-selection through the therapeutic use of other antimicrobial agents such macrolides or tetracycline, and the use metals such as copper or zinc as feed additives [42, 43, 44]. Accordingly, a high rate of phenotypic resistance to erythromycin and tetracycline was observed among the isolates in this study. Genetically, macrolide resistance encoded by *ermB* and tetracycline resistance encoded by *tet* genes, has been linked to the transposons of the Tn*1546* family that contain the *vanA* gene [42, 45, 46]. Correspondingly, *erm(B)*, and *tet(W)* were frequently detected among the isolates. Furthermore, the *E. faecium* isolated from pigs in this study contained typical adaptations to the porcine environment, such as zinc and copper resistance genes [47].

Other resistance genes identified among the *E. faecium* isolates included the *aac(6')-Ii* gene which is ubiquitous in *E. faecium* and thought to contribute to intrinsic aminoglycoside resistance [48]. Similarly, *aac*(*6*')-*Iid* which likewise is species specific [49], was found uniformly among the *E. durans* isolates in this study. *E. faecalis* is intrinsically resistant to lincosamide, streptogramins A, and pleuromutilins (LS_AP) due to the presence of *linA,* which was accordingly observed in the *E. faecalis* isolate R277 from cattle in this study. By contrast, in *E.*

faecium, LS_AP resistance is acquired by a C1349T point mutation in the Enterococcus ABC transporter gene *eat(A)* [50]. In human isolates, the mutated allelic variant *eat(A)*$_v$ has been reported in 23% of a collection of epidemiologically unrelated clinical isolates, including isolates corresponding to colonization or fecal carriage [50, 51]. In the present study, *eat*(A)$_v$ was identified uniformly in *E. faecium* from poultry and from pigs. To our knowledge, *eat(A)*$_v$ has not been described previously among porcine and poultry associated *E. faecium* isolates. Its significance as a potential marker for epidemiological studies could be investigated in future studies.

VanA-type resistance is generally mediated by Tn*1546*-like transposons that are frequently carried by self-transferable plasmids [9]. However, under the experimental conditions applied in this study, transfer of vancomycin resistance was obtained only from porcine donors. Our data confirm the possibility of *vanA* transfer from porcine *E. faecium* to human *E. faecalis*, as demonstrated previously *in vitro* and *in vivo* in the intestines of mice [52].

The Tn*1546* structures among the enterococci from this study were very similar. The majority contained the G8234T point mutation within *vanX* which has been found in enterococcal isolates from healthy and hospitalised humans, in pig isolates, in food isolates, and in environmental enterococci [53, 54, 55, 56]. This indicates that this transposon type is widely disseminated and shared between different enterococcal species and ecological niches.

Many *vanA* type Tn*1546*-like structures contain IS elements that likely play a role in the evolution of vancomycin resistance [9]. IS*1216V* is one of the main IS elements frequently observed in the *orf2*-*vanR* and the *vanX*–*vanY* intergenic regions within Tn*1546* from different sources worldwide [14, 53, 57]. The absence of IS*1216V* within the Tn*1546* elements of the isolates from this study is characteristic for VRE*fm* identified previously in strains in Europe in the late 1990s and 2000s, indicating that the *vanA*-type resistance mechanism may be very conserved among livestock enterococci in Switzerland. The lack of

the diversity observed in Tn*1546* structures which is typical for human clinical isolates suggests a limited sharing of resistance genes between livestock and human VRE, as observed previously for livestock and human enterococci isolates analysed in the UK [58].

Using cgMLST indicated that the *E. faecalis* and the *E. faecium* isolates belonged to STs typically identified among livestock-associated strains [55]. *E. faecium* isolated from pigs in this study were represented by a distinct population belonging to ST133. ST133 clusters within subgroup complex-5 (ST5) which contains STs that been identified in pigs and human hosts in Europe, including pigs from Denmark, Portugal, Spain, Switzerland, in healthy and in hospitalised patients in Denmark, Germany and Portugal, notably however, unrelated to nosocomial spread [17, 54]. These findings suggest a limited potential for transmission of VRE between humans and pigs. Similarly, *E. faecium* ST310, detected in three poultry isolates, is a poultry-adapted ST that is prevalent among broilers in Sweden [59, 60], and *E. faecium* ST13 and ST157 have been reported in poultry in Sweden, Denmark and Korea [55]. Taken together, MLST analysis did not reveal any close relationship to typical nosocomial strains belonging to CC17, or to recently emerged endemic strains ST203 and ST796. Likewise, *E. durans* is detected frequently in healthy poultry, but is reported only sporadically in human clinical infections [55]. *E. durans* of human and animal origin have been found to contain similar genetic arrangements of the *vanA* gene cluster, and it has been shown in vitro that *E. durans* transfers *vanA* to human clinical *E. faecium* at a high frequency [61, 62]. Conversely, in our study, the *E. durans* did not result in transferability to *E. faecali*s, at least under the given experimental circumstances.

Conclusions

Our study provides further evidence of the occurrence of *vanA*-type VRE in livestock, including healthy cattle, pigs, and poultry. Our results suggest that porcine *E. faecium* may be prone to transfer *vanA* genes to human related *E. faecalis*. Furthermore, our data confirm previous studies that show that there is limited sharing of livestock-associated VRE strains with strains associated with sporadic human disease, and we did not identify any clones related to hospital-related outbreak strains such as CC17.

Authors' contributions

RS designed the study. VW was responsible for sample collection. MM, KZ, SS and VW carried out the microbiological and molecular biological tests and contributed to analysis and interpretation of the data. VW, RS, SS, and MNI analyzed and interpreted the data. MJAS was responsible for bioinformatics, analysis of the WGS data, and contributed to writing the manuscript. MNI drafted the manuscript. All authors read and approved the final manuscript.

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Table 1: Phenotypic and genotypic features of vancomycin resistant *Enterococcus* spp. isolated from cattle, pigs, and poultry

		Resistance phenotype		**Resistance genotype**		
Host	**Species**	**MIC [µg/ml] vancomycin**	**Additional resistances[1]**	**Resistance genes**	**Tn1546 type**	**MLST**
Cattle						
R227	*E. faecalis*	≥ 128	–	*dfrE, emeA*, *efrA*, *efrB*, *lsaA*, *vanA*	I	29
Pigs						
Sw253	*E. faecium*	≥256	PEN, TE	*aac(6')-Ii, eat(A)*$_v$, *cadA*, *cadC*, *copZ*, *czrA*, *merA*, *merR*, *tetW/N/W*, *vanA*, *zosA*	II	133
Sw245	*E. faecium*	≥256	PEN, TE	*aac(6')-Ii, eat(A)*$_v$, *cadA*, *cadC*, *copZ*, *czrA*, *merA*, *merR*, *tetW/N/W*, *vanA*, *zosA*	II	133
Sw290	*E. faecium*	≥256	PEN, TE	*aac(6')-Ii, eat(A)*$_v$, *cadA*, *cadC*, *copZ*, *czrA*, *merA*, *merR*, *tetW/N/W*, *vanA*, *zosA*	II	133
Sw292	*E. faecium*	≥256	PEN, TE	*aac(6')-Ii, eat(A)*$_v$, *cadA*, *cadC*, *copZ*, *czrA*, *merA*, *merR*, *tetW/N/W*, *vanA*, *zosA*	II	133
Sw342	*E. faecium*	≥256	PEN, ERY, TE	*aac(6')-Ii, eat(A)*$_v$, *cadA*, *cadC*, *copZ*, *czrA*, *merA*, *merR*, *tetW/N/W*, *vanA*, *zosA*	II	133
Sw348	*E. faecium*	≥256	PEN, TE	*aac(6')-Ii, eat(A)*$_v$, *cadA*, *cadC*, *copZ*, *czrA*, *merA*, *merR*, *tetW/N/W*, *vanA*, *zosA*	II	133
Poultry						
GH14	*E. faecium*	≥256	PEN	*aac(6')-Ii, aadK, eat(A)*$_v$, *vanA*	II	157
GH24	*E. faecium*	≥256	ERY	*aac(6')-Ii, aadK, eat(A)*$_v$, *vanA*	II	310
GH32	*E. faecium*	≥256	ERY	*aac(6')-Ii, aadK, eat(A)*$_v$, *vanA*	II	310
GH58	*E. faecium*	≥256	ERY	*aac(6')-Ii, aadK, eat(A)*$_v$, *vanA*	II	13
GH76	*E. faecium*	≥256	ERY	*aac(6')-Ii, aadK, eat(A)*$_v$, *vanA*	II	310
GH98	*E. faecium*	≥256	–	*aac(6')-Ii, aadK, eat(A)*$_v$, *vanA*	II	157
GH27	*E. durans*	≥256	TE	*aac(6')-Iid, tetW/N/W*, *vanA*	III	–
GH29	*E. durans*	≥256	ERY, TE	*aac(6')-Iid, ermB tetW/N/W*, *vanA*	III	–
GH34	*E. durans*	≥256	ERY, TE	*aac(6')-Iid, ermB tetW/N/W*, *vanA*	III	–
GH44	*E. durans*	≥256	ERY, TE	*aac(6')-Iid, ermB tetW/N/W*, *vanA*	III	–

GH48	*E. durans*	256	ERY, TE	*aac(6')-Iid, ermB tetW/N/W*, *vanA*	I	–
GH50	*E. durans*	≥256	ERY, TE	*aac(6')-Iid, ermB*, *vanA*	I	–
GH61	*E. durans*	≥256	TE	*aac(6')-Iid, tetW/N/W*, *vanA*	I	–
GH63	*E. durans*	≥256	ERY, TE	*aac(6')-Iid, ermB tetW/N/W*, *vanA*	II	–
GH73	*E. durans*	≥256	ERY, TE	*aac(6')-Iid, ermB*, *vanA*	I	–
GH102	*E. durans*	≥256	TE	*aac(6')-Iid, ermB, tetW/N/W*, *vanA*	II	–

Abbreviations: *aac(6')-Ii* and *aac(6')-Iid*: genes for aminoglycoside N-acetyltransferases; *aadK,* aminoglycoside 6-adenylyl-transferase; *cad(A)*, *cad(C),* cadmium resistance genes; *cop(Z)*, copper resistance gene; *czrA*, metal transport repressor gene; *dfrE* dihydrofolate reductase gene; $eat(A)_v$, allelic variant of *eat(A)* gene for resistance to lincosamides, streptogramins A, and pleuromutilins (LS_AP); *emeA*, enterococcal multidrug resistance efflux gene; *efrA*, *efrB*, ABC multidrug efflux pump genes; *ermB*, gene for 23S ribosomal RNA methyl-transferase; *lsaA*, active efflux ABC transporter gene for intrinsic LS_AP resistance; *mer(A)*, *mer(R)*, mercury resistance genes; *tetW/N/W*, mosaic tetracycline resistance gene and ribosomal protection protein; *zosA*, zinc transporter gene.

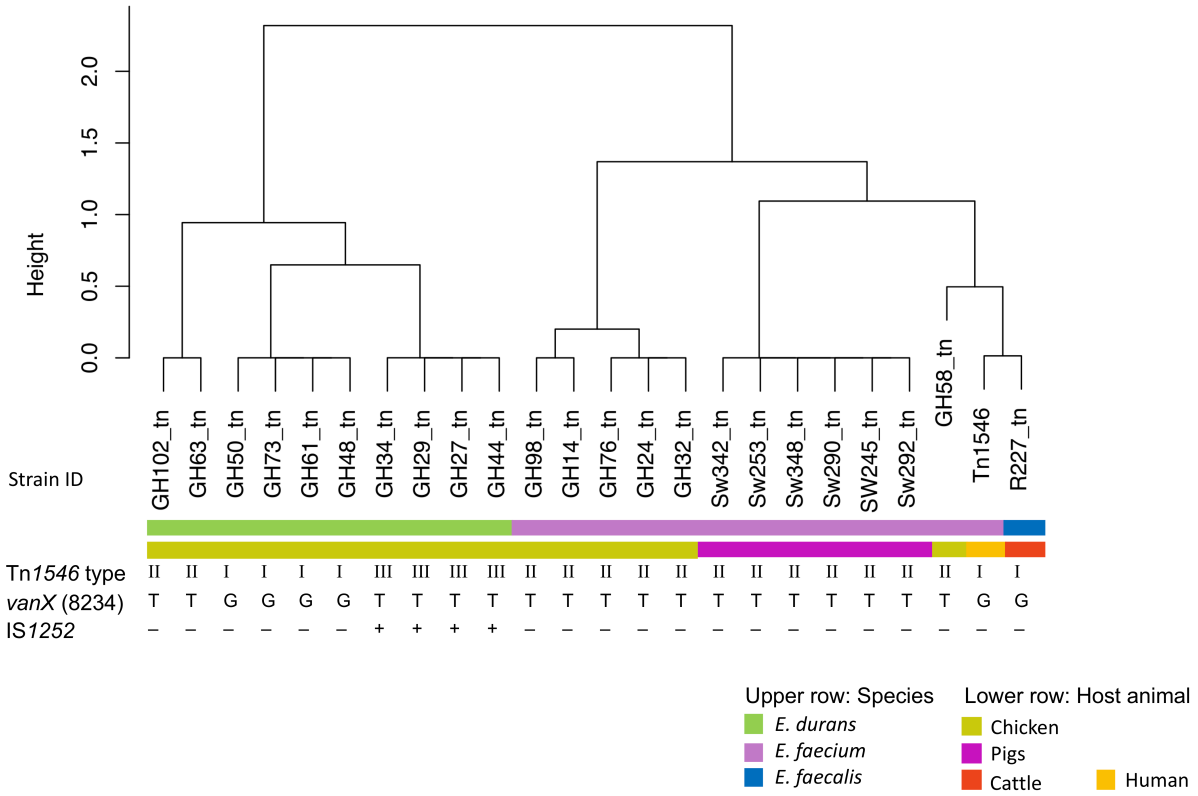

Figure 1: Average nucleotide identity (ANI) based cluster dendrogram showing three types of Tn*1546*-like elements carrying *vanA* operons identified in 23 *vanA*- type vancomycin resistant enterococci from healthy food producing animals. Type I corresponds to the prototype Tn*1546* (GenBank M97297) from human *E. faecium* B4147 [35]. Type II carries the G toT nucleotide point mutation at position 8234 in *vanX*. Tn1546-like element type III additionally carries an IS*1252* in the *orf*2-*van*R intergenic region.

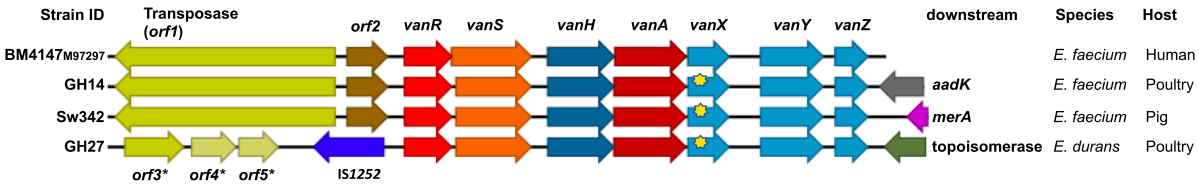

Figure 2: Linear maps of *vanA* encoding regions of the prototype Tn*1546* (GenBank M97297) from human *E. faecium* B4147 [35], and of vancomycin resistant enterococci from healthy food producing animals. Yellow stars indicate the G to T nucleotide point mutation at position 8234 in *vanX*; *aadK,* aminoglycoside 6-adenylyltransferase; *merA,* mercury resistance gene; *, putative open reading frames.

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